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ISOLATION AND DIAGNOSIS OF CADMIUM-RESISTANT BACTERIA AND ITS POTENTIAL PHYTOREMEDIATION WITH THE BROAD BEAN PLANT

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SUMMARY

Results of the study proved that phytoremediation can be a promising technique to treat cadmium (Cd)-contaminated soil. Four bacteria types were isolated from the soil; two are autotrophic and others are heterotrophic. Autotrophic bacteria were dominant in soils with 42 mg Cd Kg^{-1}. The total count and diversity of both bacteria types decreased with the increase of Cd in media and reached their minimum limit of tolerance at 60 mg Cd L^{-1} in terms of the heterotrophic bacteria, while the minimum limit of tolerance in the case of autotrophic bacteria was at 110 mg Cd L^{-1}. The four isolates can form biofilms that ranged in thickness between 2.8–4.3 mm. The tolerant isolates belong to *Rhizobium leguminosarum, Pseudomonas fluorescens, Actinobacteria, and Corynebacterium*. Shoot and dry weight significantly varied according to the changes in Cd concentrations and isolate types. The level in either shoot or root exceeded critical levels, however, its concentration was higher in the root compared with the shoot. The effect of Cd on broad bean plants began at 80 and 100 mg Cd L^{-1}. The broad bean plant was resistant to growing in the contaminated area by Cd even at 120 mg Cd Kg^{-1} DW. The presence of heterotrophic bacteria was noticeably useful for autotrophic bacteria, as well as, for enhancing Cd resistance. The study showed that cooperative phytoremediation could be a safe and active technique to apply in the field soil contaminated with heavy metals.

Keywords: Broad bean, cadmium, heavy metals, pollution, resistant bacteria

Key findings: The significance of this research is to highlight the role of bacteria to resist cadmium stress in polluted soils, and more importantly the synergistic cooperation between crops and bacteria to cope with pollutants.

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INTRODUCTION

The pollution of soils and crops with cadmium (Cd) is considered an environmental problem in each area where extensive agricultural practices take place. Cadmium (Cd) is a toxic metal and forms a threat to soil quality, food safety, and human health due to its toxicity and solubility in water. The absorbed Cd affects plant growth and assimilation. Recently, studies focused on the accumulation of Cd in the environment and its relation with biological

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activities and plant production. The various biological systems were used to monitor the environment and watch the accumulation of pollutants and some of those systems were used to aggregate and remove pollutants (e.g. Cd) (Agunbiade and Fawale, 2009).

The presence of Cd in soil negatively affects plant cells through changing assimilation by entering the metabolism pathways. Also, Cd damage plastids and mitochondria, as well as, increase the oxidative of lipids and proteins. On the other hand, Cd competes with the absorption and transfer of other important elements, such as, P and K, eventually leading to a reduction in plant growth and production (Gómez-Sagasti and Marino, 2015; Hanafiah *et al*., 2021). Blaylock and Huang (2000) clarified that heavy metals in soil ranged between 1 to 100 mg Kg^{-1}, and this wide range is due to origin participation or through human practices and industrial activities. However, Miller (1996) and Ebbs and Kochian (1997) findings indicated that wet soil that contains 10 mg Cd Kg^{-1} is considered contaminated soil, while in dry soil the range could reach 25 mg Cd Kg^{-1}.

The fixation of Cd in soil by using organic modifications is an active and environment-friendly method. In addition, it is cost-effective and can be used in moderately to highly contaminated soils. Cabrera *et al*. (2006) developed a biological engineering model that uses bacteria in the resistance and removal of Cd from plants, and succeeded in the fast removal of 24.70% from 15 mg Cd L^{-1} in the media. Majer *et al*. (2002) findings revealed that the increase of Cd concentration in the environment affects the biological activities and confirmed the possibility of using microorganisms in Cd removal from contaminated soil and water. The understanding of plant cells' ability to accumulate and transfer pollutants is a very important way to investigate their removal from the environment. It has been found that Cd-resistant bacteria were *Bacillus* and *Pseudomonas* whose resistance reached 225 and 275 mg Cd Kg^{-1} from soluble Cd, respectively. Those two types are widely available in soil and water, and can form biofilms that increase their stickiness between themselves, with stone or metals (Donlan and Costerton, 2002).

Heavy metals stress leads to a reduction in bacterial biodiversity, biomass, and its activities. Microorganisms, such as, bacteria cannot grow in the presence of high concentrations. Removal or treating soil pollutants is a very big challenge. Physical, chemical, and biological techniques were used to treat pollutants in soils, such as, heavy metals. Some bacteria and fungi were found to be very efficient in treating contaminated soil and water (Pane *et al*., 2008; Gómez-Sagasti and Marino, 2015; Krisnawati *et al*., 2019). The symbiotic relation of plant and bacteria, and some auto and heterotrophic isolates, appeared to have a significant role in pollutants removal from soils. There is an increasing interest in understanding the symbiotic relation between legumes and rhizobia and its interaction with Cd (Rodrigues *et al*., 2019).

Based on the aforementioned information, this study was conducted to evaluate the presence of microbes with Cd and assess the broad bean (*Vicia faba* L.) plants' tolerance to it. Moreover, the main effects of Cd on the legume crop on atmosphere nitrogen fixing, and how to utilize this symbiotic relation to actively remove Cd from contaminated soils, were also studied.

MATERIALS AND METHODS

Composite soil samples were collected in June 2018 from three locations of sewerage dumps in Ramadi city (Anbar Governorate, Iraq) at the depth of 0–30 cm. The samples were taken in sterilized, clean, and shaded cylinders, and then transferred to the laboratory for further investigations. Cadmium concentration in the samples was estimated by digestions, using the atomic absorption spectrophotometer (EMC-11 UV spectrophotometer - Germany). The soil samples were described according to the microorganisms by isolating the Cd-resistant bacteria. Heterotrophic and autotrophic bacteria were counted along with their diversity in the soil through the testing of colonies growth on agar medium of soil extract supported with 1% glucose and inoculated with diluted soil extract using the pour plate method.

Heterotrophic bacteria numbers were counted and colony types were repeated in more than five types in one plate (Hobbie *et al.*, 1977). The same method was used in the case of autotrophic bacteria in the medium of Difco, Baltimore, MD: 0.5 g yeast extract; 0.5 g proteose peptone; 0.5 g casamino acids; 0.5 g dextrose, $C_6H_{12}O_6$; 0.5 g soluble starch, $(C_6H_{10}O_5)n$; 0.3 g sodium pyruvate, $NaC_3H_3O_3$; 0.3 g potassium phosphate, K_2HPO_4; 0.05 g magnesium sulfate, $MgSO_4$; and 15 g agar, pH 7.2. Plates were incubated for one week at 25 °C. The media were inoculated with a series of dilutions of soil extracts and colony types were repeated more than five times and were recorded according to Hobbie *et al*. (1977).

The toxicity level of Cd and the resistance of the isolations were evaluated by exposing isolated bacteria from soil—grown in the medium: 0.5 g sodium citrate, $C_6H_5Na_3O_7$; 0.1 g magnesium sulfate, $MgSO_4$; 1.0 g ammonium sulfate, $(NH_4)_2SO_4$; 1.0 g glucose, $C_6H_{12}O_6$, and 0.1 g sodium pyrophosphate, $Na_4P_2O_7(H_2O)10$, buffered to pH 6.0 with potassium phthalate, ($KHC_8H_4O_4$) to Cd concentrations of 40, 50, 60, 70, 80, 90, 100, and 110 mg Cd L^{-1} (Soluble $CdCl_2$). Mineral media (20 mL) was inoculated with 1 mL from diluted soil extract (1:10). The bacterial growth was observed every 24 h for seven days until colonies reached to stage of stability. To determine the actual Cd-resistant bacteria numbers, 1 mL on the same medium was provided with the same Cd concentrations to obtain the Cd-resistant isolations (autotrophic and heterotrophic).

Bacterial traits and descriptions were studied on the selected Cd-resistant isolations including Gram-positive and negative, biochemical properties, such as, SOD and CAT, and hydrolysis of starch and sugars. The isolations were diagnosed by SrRNA16 sequencing techniques (Andreou, 2013). A method described by Sauer *et al*. (2002) was used to investigate the ability of isolates to resist Cd and produce plasma membrane. Isolates were grown in 10 mL tubes containing Trypticase Soy Broth and nutrient broth, besides the negative control, and incubated for 24 h at 37 ºC. Tubes content was poured and 10 mL from safranin stain (0.1%) was added and left for 1 min, then poured and flipped. The results were recorded based on the function of their color. The formed membrane thickness was also estimated using the classical method of Sauer *et al*. (2002).

The isolates belonging to strains of *Ps. fluorescens*, *R. leguminosarum*, *Actinobacteria, and Corynebacterium* were tested for their ability for to remove Cd individually or collectively, whether they were autotrophic or heterotrophic. The soil extract medium (1 soil: 5 water) was used in 500 ml flasks and provided with 250 ml containing 20, 40, 60, 80, 100, and 120 mg Cd L^{-1} for three replicates of each concentration, and the pH was adjusted to 7.2. Treatments were inoculated with 10^6 CFU mL^{-1} and broad bean (cv. Otono De Luz) seeds were planted under lab conditions using a piece of cloth, and incubated for 45 days at 28 °C. A factorial randomized complete block design was used and the following traits were calculated: plant height, root length, number of nodules, dry weight, microbial content, and Cd concentration in plant and solution.

RESULTS

Cadmium-contaminated soils in the three locations

Results of soil analysis showed that it contained 42 mg Cd Kg^{-1} soil and the dominance of autotrophic bacteria (1.2×10^7 CFU g^{-1}). There were 12 types of colonies that repeated more than 5 times. However, heterotrophic bacteria reached 5.1×10^4 CFU g^{-1} and four types were observed that repeated more than 5 times.

The data showed that evaluation of Cd toxicity and isolate resistance to Cd among autotrophic and heterotrophic bacteria were separated from contaminated soils (Table 1). It

Table 1. Isolated bacteria resistance to Cd concentration in media.

Bacteria based on nutrition	Diversity and density	Cadmium concentration in media mg L^{-1}							
		40	50	60	70	80	90	100	110
Autotrophic	TM	4.2×10^7	4.9×10^6	1.5×10^6	2.3×10^5	1.2×10^5	3.6×10^4	2.2×10^3	1.3×10^2
	Type	10	8	6	5	4	3	2	2
Heterotrophic	TM	5.6×10^4	3.3×10^3	2.1×10^2	0	0	0	0	0
	Type	4	3	2	0	0	0	0	0

has also shown the superiority of autotrophic bacteria in number and diversity over heterotrophic bacteria in their resistance to Cd in the media. The total microbe (TM) reached 4.2×10^7 CFU g^{-1} with an overall diversity of 10 in autotrophic bacteria, whereas with the heterotrophic bacteria, TM got to 5.6×10^4 CFU g^{-1} with onlyfour types. The total number and diversity of both bacterial types were decreased as the Cd concentrations increase in the media. The maximum concentration that heterotrophic bacteria can tolerate is 60 gm Cd L^{-1}, whereas the autotrophic bacteria were still able to grow in the media containing 110 mg Cd L^{-1} (1.3×10^2 TM, two types). The autotrophic bacteria are more resistant to the increase in cadmium in media in comparison with heterotrophic bacteria. Diagnosis results showed that autotrophic and heterotrophic bacteria belong to *R. leguminosarum, P. fluorescens, Actinobacteria,* and *Corynebacterium*.

The ability of isolates for forming biofilms

The tubes used in testing the biofilms by isolated bacteria showed that all bacteria formed a sticky red layer (biofilm). The intensity of the red color increased with the increase of the thickness of this layer. The isolates (four isolates) varied in their efficiency in forming those films. The biofilm thickness was 4.4, 3.8, 2.6, and 2.3 mm in autotrophic bacteria isolates *Corynebacterium* and *Actinobacteria,* and heterotrophic bacteria *P. fluorescens* and *R. leguminosarum,* respectively. The variance in biofilm thickness might belong to physiological and genetic factors.

Effects of Cd and bacterial isolates on broad bean

After 45 days of planting, the root length has been significantly decreased with the increase in Cd concentrations in the medium (Figure 1). They reached the lowest level in the media when treated with 120 mg Cd L^{-1}. The data also showed that root growth was affected more than shoot growth. The highest plant height and root length were recorded when media contained 40 mg Cd L^{-1}. It also showed that the plant height and root length reached their maximum values in the media treated with the mixture of auto and heterotrophic bacteria ($p<0.05$), and followed by media treated with autotrophic bacteria. The role of bacterial treatments (autotrophic and heterotrophic) was highlighted here by showing that treated plants with bacteria showed higher growth in both root and shoot. Data indicated the two-way interaction between bacterial treatments and the application of Cd had a significant effect on the shoot and root growth, shoot and root dry weight, number of nodules per plant, Cd concentration in shoot and root, and Cd residues in media (Table 2). It further revealed that the interaction between Cd concentration and bacterial treatments had a significant effect on plant height and root length of broad bean plants after 45 days from planting. The highest mean of plant height and root length (35 and 16.4 cm) were recorded with the concentration of 40 mg Cd L^{-1} and treated with a mixture of bacteria and autotrophic bacteria, respectively, whereas the lowest values (12.1 and 12.4 cm) were obtained at 120 mg Cd L^{-1} in the control, and when the media is treated with heterotrophic bacteria, respectively. Results proved that Cd harmed plant height and root length and treating media with a mixture of autotrophic and heterotrophic bacteria, followed by treating media with autotrophic bacteria and heterotrophic bacteria increased plant resistance to Cd (Figure 1, Table 2). However, the lowest values were recorded in control treatment especially when 120 mg Cd L^{-1} was added to the media.

The nodules formation had been significantly affected by increasing Cd concentration in media (Figure 2). The highest number of nodules formed on broad bean root was recorded in media treated with 40 mg Cd L^{-1}, then decreased gradually as the Cd concentration increased in the media. Figure 2 also indicated that the use of a mixture of auto and heterotrophic bacteria had a highly significant effect on the number of nodules on plant roots, followed by using the heterotrophic bacteria in media, which gave the highest numbers of formed nodules. On the other hand, using autotrophic bacteria and control treatment gave the lowest number of nodules per plant and significantly differed from the other two treatments. The two-way interaction between Cd and bacterial treatments indicated a significant effect on the number of nodules per plant. It is clear that the use of either heterotrophic bacteria or a mixture of the two types of bacteria showed higher nodules formed on the root, however, all treatments were close with the increase of concentration. The number of nodules reached 22.8 and 22.4 (Table 2) when a mixture of isolates and heterotrophic bacteria were used, respectively. However, in the treatments of the control and autotrophic bacteria, there were no nodules

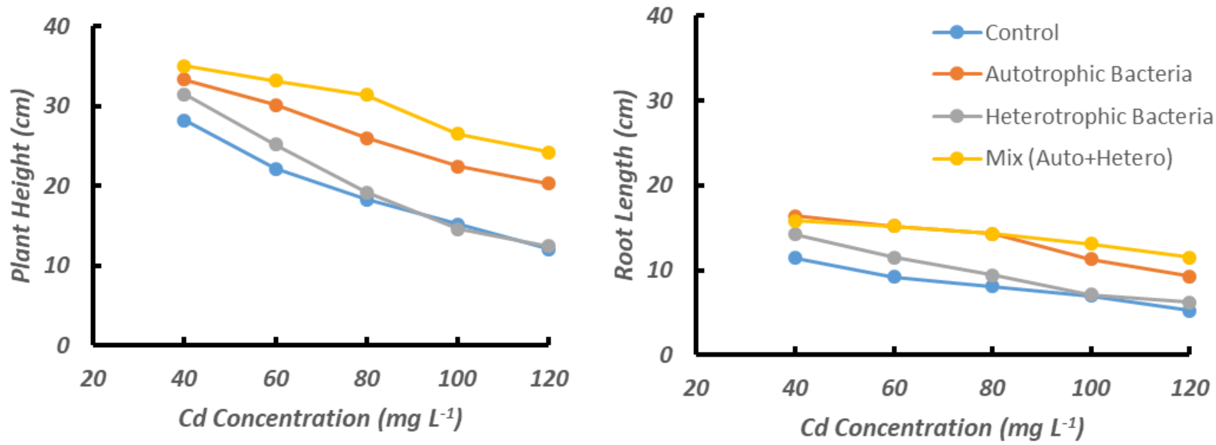

Figure 1. Effect of cadmium in media treated with auto, hetero, and the mixture of them on the plant height (right) and root length (left) of broad bean plant ($LSD_{0.05}$ = Shoot: Cd = 3.45, Bacteria = 2.13, Root: Cd = 2.24, Bacteria = 1.83, n=3).

Table 2. Effect of Cd and bacterial treatments on some broad bean traits.

Bacterial Treatments	Cd mg L^{-1}	Plant Height (cm)	Root Length (cm)	Shoot DW (g)	Root DW (g)	Number of Nodules $plant^{-1}$	Cd in Shoot (mg g DW^{-1})	Cd in Root (mg g DW^{-1})	Residues of Cd in media mg L^{-1}
Control	40	28.2	11.4	10.2	3.4	4.2	12.8	35.2	30.5
	60	22.1	9.2	8.1	2.3	2.3	22.4	48.6	48.6
	80	18.3	8.1	6.2	1.9	1.2	38.2	53.4	72.4
	100	15.2	7	4.1	1.3	0	52.4	69.3	91.3
	120	12.1	5.2	4	1.1	0	60.5	78.8	110
Autotrophic Bacteria	40	33.3	16.4	13.4	5.2	6.2	8.2	16.2	12.3
	60	30.1	15.2	13.2	4.6	3.5	11.7	20.4	18.5
	80	26	14.3	12.1	3.8	3.3	14.6	28	25.2
	100	22.4	11.3	8.2	2.5	0	18.4	37.1	36.3
	120	20.3	9.3	6.1	2	0	22.4	41.5	43.4
Heterotrophic Bacteria	40	31.5	14.2	14.5	5.2	22.4	7.3	20.4	15.5
	60	25.2	11.5	12.3	3.8	16.6	14.3	32.3	21.4
	80	19.2	9.4	8.4	2.2	8.4	22.6	46	48.5
	100	14.6	7.1	4.3	1.4	4.2	33.5	52.1	53.2
	120	12.4	6.2	4	1.2	1.4	48.5	60.6	60.3
Mix (Auto+ Hetero)	40	35	15.8	14.6	5.8	22.8	5.4	9.5	8.3
	60	33.2	15.2	14.3	4.7	18.3	6.4	13.3	12.6
	80	31.4	14.3	12.6	3.9	12.5	8.6	18.4	16.6
	100	26.5	13.1	10.2	3.1	6.6	12.3	26.2	20.4
	120	24.2	11.5	8.1	2.6	4.4	15.5	30.4	28.5
$LSD_{0.05}$		4.62	3.52	3.64	3.32	3.66	4.92	6.12	8.6

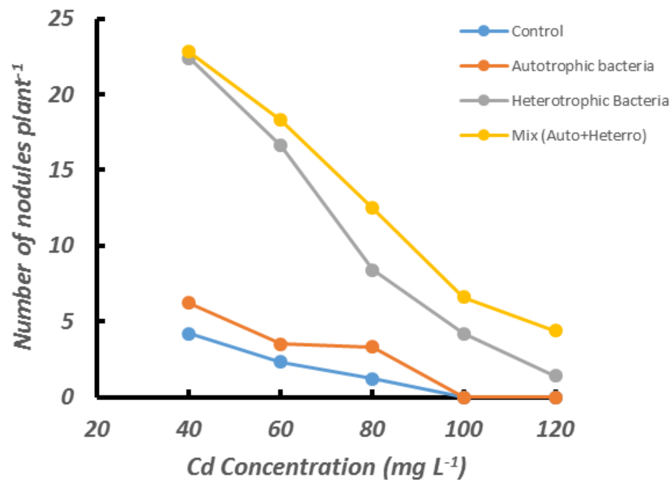

Figure 2. Effect of cadmium in media treated with auto, hetero, and the mixture of them on several nodules formed on the root of broad bean plant ($LSD_{0.05}$: Cd=2.47, Bacteria= 2.73, n=3).

formed on the roots, especially at 100 and 120 mg Cd L^{-1}.

The dry weight of broad bean shoot and root was significantly decreased with the increase of Cd concentration in media. The highest values of dry weight were recorded when only 40 mg Cd L^{-1} was added to the media, then the weight gradually declined to reach its lowest average when the media was treated with a high concentration of Cd (100 and 120 mg Cd L^{-1}) (Figure 3). It also indicated that plants treated with either autotrophic bacteria or with a mixture of auto and heterotrophic bacteria showed the highest values of dry weight in shoot and root in comparison with the other two treatments. It also appeared that the root was significantly more affected by Cd than the shoot.

Significant interactions were recorded between Cd concentrations and bacterial treatments (Table 2). The highest average of shoot dry weight, i.e., 14.60 and 14.50 g $plant^{-1}$ was recorded when the media were treated with 40 mg Cd L^{-1}, and with the use of either mixture of isolated or heterotrophic bacteria, respectively, whereas the lowest averages ranged between 4.00–4.30 g $plant^{-1}$, obtained when the media was treated with 100 and 120 mg Cd L^{-1} for control and with heterotrophic treatments, respectively. It seems that broad bean roots behaved similarly in terms of dry weight in comparison with shoot dry weight. The highest averages of root dry weight ranged between 5.20–5.80 g $plant^{-1}$ where auto and heterotrophic bacteria and their mixture were used on the media treated with 40 mg Cd L^{-1}, respectively. In contrast, the lowest averages ranged between 1.10 and 1.40 g $plant^{-1}$ were respectively obtained when media treated with 100 and 120 mg Cd L^{-1} for the control and with heterotrophic bacteria only.

The concentration of Cd in the shoot and root of the broad bean was also investigated in this study. The absorption of Cd was enhanced with the increase of its concentration in the media reaching the highest level when media was treated with 120 mg Cd L^{-1}, whereas the lowest concentration of Cd was recorded at 40 mg Cd L^{-1} in the shoot (Figure 3). The accumulation of Cd in the root also increased with the increase of Cd in the media. The accumulation of Cd in the root was slightly higher than its concentration in the shoot. The use of bacterial treatments in the media significantly reduced the absorption of Cd by the plant (Figure 3). The availability of auto and heterotrophic bacteria in the media led to less Cd concentration in shoot and root of broad bean plant, followed by autotrophic bacteria alone, then heterotrophic bacteria alone in comparison with the control treatment. In addition, the two-way interaction between Cd and bacteria treatments significantly affected the concentration of Cd in both shoot and root. The lowest concentrations of Cd (5.40 and 9.50 mg g DW^{-1}) were recorded in the plant shoot and root grown in the media containing 40 mg Cd L^{-1} and treated with a mixture of auto and heterotrophic bacteria (Table 2). However, the highest levels of Cd (60.50 and 78.80 mg g DW^{-1}) were observed in a plant grown in the media containing 120 mg Cd L^{-1} and free of bacteria.

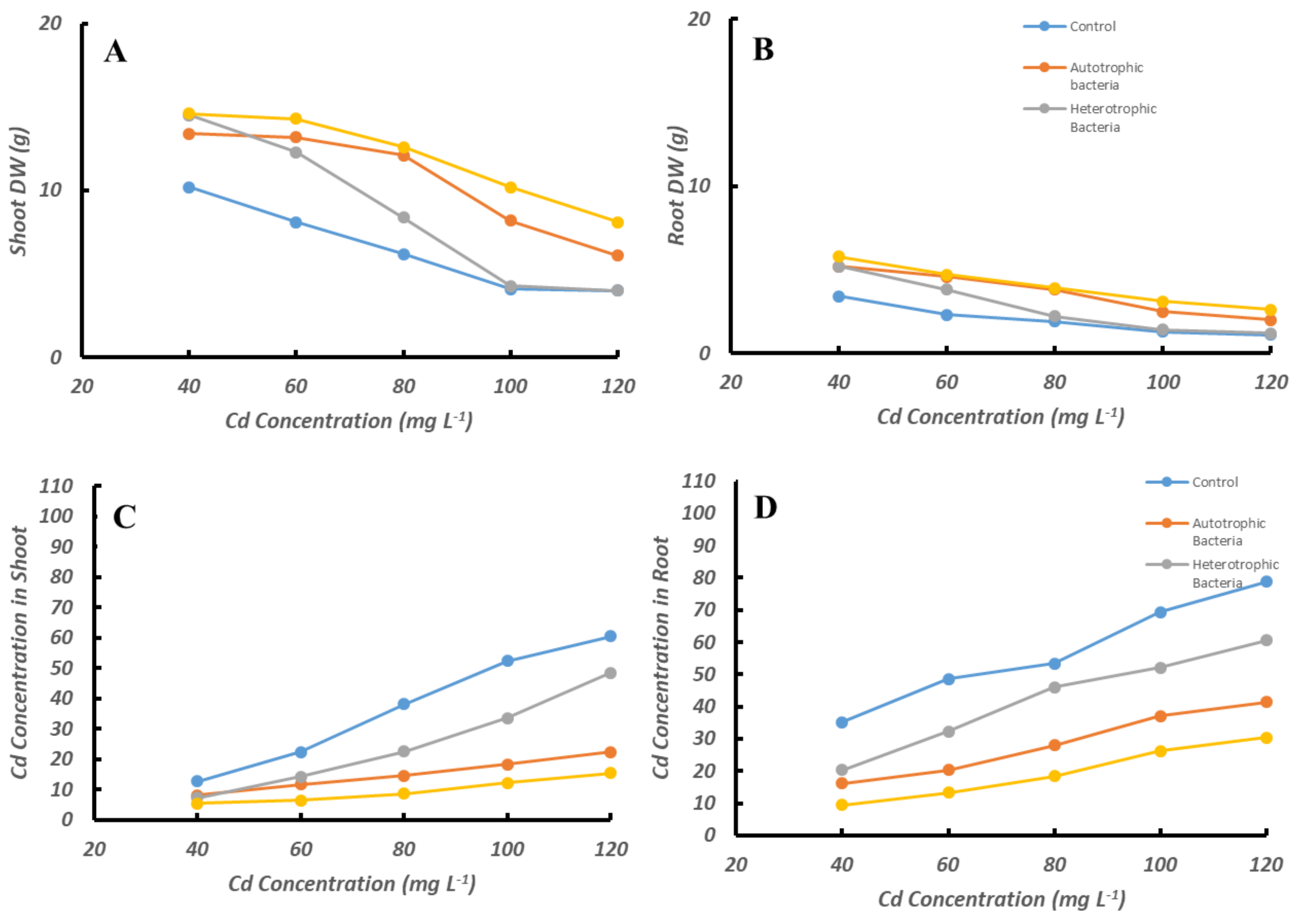

Figure 3. Effect of cadmium in media treated with auto, hetero and mixture of them on shoot DW (A), root DW (B) Cd concentration in shoot (C) and Cd concentration in root (D) ($LSD_{0.05}$= Shoot DW: Cd=1.44, Bacteria= 2.23, Root DW: Cd= 1.64, Bacteria= 1.23, Cd conc in Shoot: Cd=3.64, Bacteria= 2.53, Cd conc in root: Cd=4.34, Bacteria= 3.15, n=3).

Cadmium residues in media by treating with bacteria and plant

Results indicated the increase of soluble Cd concentration in the media with the increase of its application to reach its top value at 120 mg Cd L^{-1}, whereas the lowest Cd residues were in the media treated with 40 mg Cd L^{-1} (Figure 4). On the other hand, bacterial isolates showed a significant effect on soluble Cd in the media, where the use of the mixture of auto and heterotrophic bacteria led to less Cd in the media, in comparison with the control treatment which contained 70.56 mg Cd L^{-1}. The two-way interaction between Cd and bacterial treatments significantly affected the soluble Cd in the media. The lowest concentration of Cd in the media was found (8.30 mg Cd L^{-1}) at 40 mg Cd L^{-1} with a mixture of isolates treatment. The highest concentration of soluble Cd was found in the control treatment when treated with 120 mg Cd L^{-1} (Table 2).

DISCUSSION

One of the growing serious problems recently is the pollution with heavy metals, where soil is considered polluted when it is affected by the high concentration of metals through emissions from the rapidly expanding industrial sector, disposal of high metal waste, pesticides, and sewage sludge. The Cd was found to be as highly toxic to plants, humans, and all living organisms. It has biological activity in terrestrial and aquatic organisms.

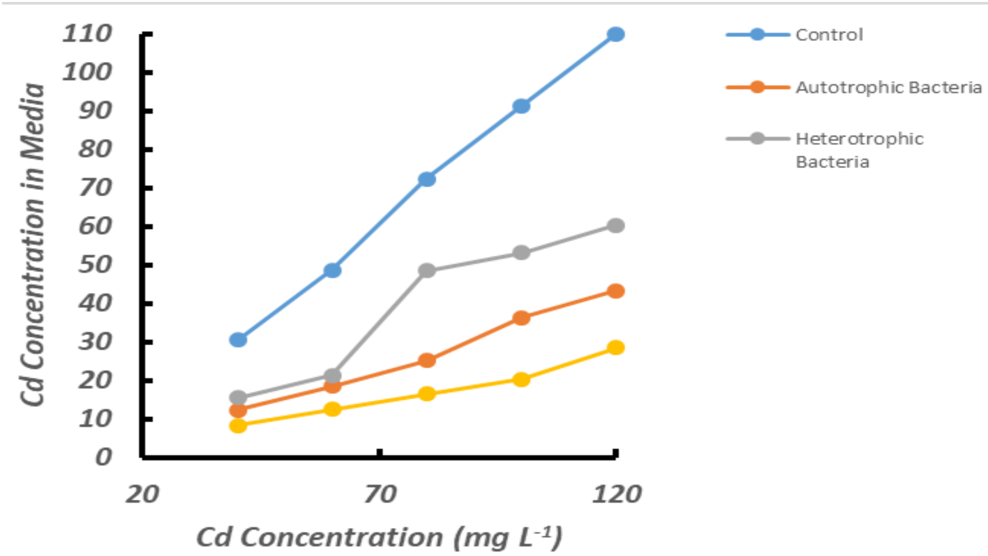

Figure 4. Effect of cadmium in media treated with auto, hetero, and the mixture of them on residues of Cd in media at the end of the experiment (LSD0.05: Cd=5.74, Bacteria=4.23, n=3).

Development in agriculture and industries has culminated in a higher concentration of Cd in agricultural soils. Cadmium enters ecosystems via numerous human activities and emissions to the environment. The admission of Cd in plants in Cd polluted soil poses severe problems to living organisms due to its high mobility in contaminated soils. Toxic pollutants, such as, cadmium have to be eliminated from the food chain, therefore phytoremediation could be a successful mitigation measure to clean heavy metal-polluted soil at less cost.

The study came to prove the efficiency of phytoremediation and to add a better understanding of the mechanisms underlying the accumulation of heavy metals in plants in the presence of bacteria. The function of the soil ecosystem depends heavily on soil microbes as they can promote material circulation, nutrient transformation, energy flow, organic matter decomposition, and other ecosystem-related biochemical processes (Lozupone *et al*., 2007; Luo *et al*., 2019). Besides their role in promoting growth, bacteria can help the plant to cope with various abiotic stresses, such as, drought, and resistance to the pathogen and heavy metals. Detecting changes in microbial populations and activities is generally more feasible than directly evaluating the physicochemical properties of soil, as the identification of soil microbes may contribute to the evaluation of remediation treatment (Luo *et al*., 2019). When soils are treated with these microbes, they reduce heavy metal uptake by reducing their availability. The low availability of these metals in rhizospheres, in turn, boosts root elongation and enhances plant growth. The metal-resistant microbes may immobilize heavy metals through the production of exopolysaccharides, siderophores, and metal phosphates or enhanced rhizosphere acidification.

The soil samples that were taken from three sewerage dump locations and contaminated with Cd showed that Cd concentration exceeded the critical level (3.99 mg kg^{-1}) (Pane *et al*., 2008). Most importantly the diagnosed bacterial isolates were found to be acclimated to the high concentration of Cd. Tolerance mechanisms in microorganisms toward specific metals often include the binding of metals by cell wall or by proteins and extracellular polymers, formation of insoluble metal sulfides, volatilization, and enhanced export from the cell (Giller *et al*., 1998; Bhartiya *et al*., 2012). The microbes also influence the metal availability by changing the pH, metal valence, chelation, and other mechanisms (Francis and Dodge, 1990). In addition, Gadd (2004) showed that microorganisms have several resistant mechanisms that can prevent heavy metal toxicity either by inducing the development of tolerance or resistance.

In general, heavy metal resistance mechanisms are an active metal efflux, synthesis of metal-binding peptides, proteins, or polysaccharides, such as. metallothioneins, extracellular polymeric substances, and the increasing detoxification enzymes expression. Heavy metal resistance is known to occur in many bacterial genera. Bacteria use various types of resistance mechanisms in response to heavy metal toxicity. The autotrophic bacteria were more resistant to Cd concentration in comparison with heterotrophic bacteria. The biodiversity of bacteria decreased with the increase of Cd in the media and that confirmed few efficient isolates did the function.

The presence of a mixture of auto and heterotrophic isolates in the media made the ability to resist more than being alone. This confirmed that isolates have various mechanisms to protect themselves against the toxicity of Cd and one can utilize the other in the media (Gómez-Sagasti and Marino, 2015). Regarding the accumulation of Cd in broad bean plants, the data of this study indicated that there were significant differences in shoot and root dry weight. All collected samples exceeded the critical level (1.5 mg Kg^{-1}). In some treatments, the concentration of Cd in the root was more than its concentration in the shoot by double or even more and this confirmed that Cd exceeded the critical level in the root (0.1 mg Kg^{-1}). It has been observed that the maximum limit of effect on broad bean traits was at 80 and 100 mg Cd L^{-1}. Data also indicated that Cd accumulated more in root over the shoot (R/S) and this elucidates the inefficiency of plants in transferring Cd from root to shoot (Liu *et al*., 2019). It has been confirmed that the broad bean plant's ability to tolerate and accumulate a high concentration of Cd reached 120 mg Cd L^{-1} (Rodrigues *et al*., 2019; Ullah *et al*., 2020).

The study results also confirmed that the comprehension of cell ability to accumulate and transfer pollutants is very important to establish the way to remove them from the environment and this was consistent with past studies by obtaining a Cd removal percentage of 88% (Mittal *et al*., 2004; Pane *et al*., 2008). The removal of Cd from media percentage was decreased with the increase of Cd in it and this result is in agreement with what has been found by Cabrera *et al*. (2006) who proved that bacteria can remove Cd from the media, but the ability decreased with the increase of Cd. This also confirmed that heavy metals at their concentrations are very important for microorganisms to complete their life cycle, and also can be a threat to their life, as well as, to the ecosystem (Ebbs and Kochian, 1997). Generally, the primary mechanism of resistance to heavy metal ions in prokaryotes is a reduced accumulation based on the active efflux to export toxic metals outside the cell (Hynninen *et al*., 2009).

CONCLUSIONS

Utilization of the microorganisms as phytoremediation of heavy metals (Cd) is a very suitable method. Selection of the appropriate Cd-resistant bacteria could be an effective mechanism to remove the toxic effect, yet depending on the level of heavy metal stress. It has been observed that when bacteria were exposed to the high concentration of Cd, their number and diversity were decreased. This is a very interesting observation because it confirmed that some bacteria have multiple mechanisms to tolerate the same metal. The mechanisms included producing a layer of polysaccharides or biofilms surrounding the cell and isolating the metal and preventing it from penetrating cells. In addition, it can produce certain proteins conjugated with Cd actively inside the cells to delay its interactions in cellular processes. This can be very important in treating the affected ecosystems. This could lead us to a clear understanding of how microorganisms respond to their environment. Finally, the mechanism can be very effective with the integration of auto and heterotrophic bacteria.

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